OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

MicroElute® Cycle-Pure Kit

D6293-00	5 preps
D6293-01	50 preps
D6293-02	200 preps

MicroElute® Gel Extraction Kit

D6294-00	5 preps
D6294-01	50 preps
D6294-02	200 preps

MicroElute® DNA Clean Up Kit

D6296-00	5 preps
D6296-01	50 preps
D6296-02	200 preps

April 2013

For research use only.Not intended for diagnostic testing.

MicroElute®Cycle Pure Kit
MicroElute® Gel Extraction Kit
MicroElute® DNA Clean Up Kit

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Introduction and Overview

The E.Z.N.A.® family of products is an innovative system that radically simplifies the extraction and purification of nucleic acids from a variety of sources. The key to this system is the HiBind® matrix that specifically, but reversibly, binds DNA or RNA under optimized conditions allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or a low salt buffer.

The MicroElute® Clean Up system, designed for rapid DNA clean up, includes:

- MicroElute® Cycle-Pure Kit - for direct purification of double or single stranded PCR products (100 bp - 10 kb) from amplification reactions
- MicroElute® Gel Extraction Kit - for extraction of DNA fragments (70 bp - 20 kb) from standard, or low-melt agarose gels in TAE (Tris-acetate/EDTA) or TBE (Tris-borate/EDTA) buffer
- MicroElute® DNA Clean Up Kit - for general clean up of oligonucleotides and DNA up to 10 kb in size from enzymatic reactions (e.g., labeling, dephosphorylation, restriction, and tailing)

Binding Capacity:
Each MicroElute® DNA Mini Column can bind ~10 µg of DNA.

New in this Edition:

- This manual has been edited for content and redesigned to enhance user readability.
- Equilibration Buffer is no longer included with this kit. An optional Column Equilibration Protocol has been added to the protocol for your convenience.
- Equilibration Buffer is replaced with 3M NaOH provided by the user.

Kit Contents

Product	D6293-00	D6293-01	D6293-02
Purifications	5	50	200
MicroElute® DNA Mini Columns	5	50	200
2 mL Collection Tubes	5	50	200
CP Buffer	5 mL	30 mL	120 mL
Elution Buffer	1 mL	15 mL	15 mL
DNA Wash Buffer	1.5 mL	15 mL	3 x 25 mL
User Manual	✓	✓	✓

Product	D6294-00	D6294-01	D6294-02
Purifications	5	50	200
MicroElute® DNA Mini Columns	5	50	200
2 mL Collection Tubes	5	50	200
Binding Buffer (XP2)	5 mL	30 mL	120 mL
Elution Buffer	1 mL	15 mL	15 mL
SPW Wash Buffer	2 mL	25 mL	3 x 25 mL
User Manual	✓	✓	✓

Product	D6296-00	D6296-01	D6296-02
Purifications	5	50	200
MicroElute® DNA Mini Columns	5	50	200
2 mL Collection Tubes	5	50	200
DP Buffer	2 mL	20 mL	80 mL
Elution Buffer	1 mL	15 mL	15 mL
SPW Wash Buffer	2 mL	25 mL	3 x 25 mL
User Manual	✓	✓	✓

Preparing Reagents

Prepare the appropriate wash buffer as directed below depending on the kit ordered.

- Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D6293-00	6 mL
D6293-01	60 mL
D6293-02	100 mL

- Dilute SPW Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D6294-00	8 mL
D6294-01	100 mL
D6294-02	100 mL per bottle

- Dilute SPW Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D6296-00	8 mL
D6296-01	100 mL
D6296-02	100 mL per bottle

Storage and Stability

All of the MicroElute® Kit components are guaranteed for at least 12 months from the date of purchase when stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in CP Buffer. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Guidelines for Vacuum Manifold

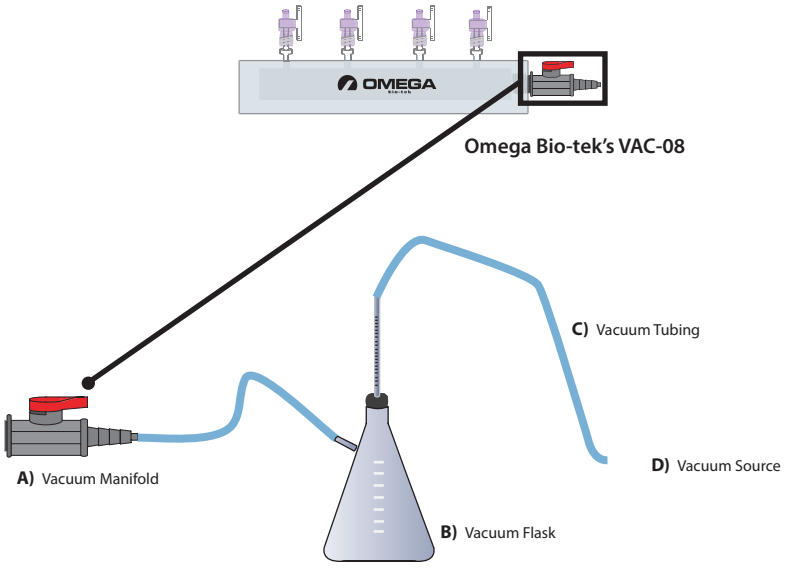
The following is required for use with the Vacuum/Spin Protocol:

A) Vacuum Manifold (We recommend Omega Bio-tek's VAC-08)
Other Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma AldrichVM20, Promega Vacman®, or manifold with standard Luer connector
B) Vacuum Flask
C) Vacuum Tubing
D) Vacuum Source (review tables below for pressure settings)

Manifold	Recommended Pressure (mbar)
VAC-08	-200 to -600

Conversion from millibars:	Multiply by:
Millimeters of mercury (mmHg)	0.75
Kilopascals (kPa)	0.1
Inches of mercury (inch Hg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per Square Inch (psi)	0.0145

Vacuum Setup:



MicroElute® Cycle-Pure Kit - Centrifugation Protocol

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x *g*
- 100% ethanol
- 1.5 mL microcentrifuge tubes
- Vortexer
- Optional: 3M NaOH
- Optional: Sterile deionized water or TE Buffer

Before Starting:

- Prepare DNA Wash Buffer according to "Preparing Reagents" section on Page 4.

1. Perform agarose gel/ethidium bromide electrophoresis to analyze PCR product.

2. Determine the volume of your PCR reaction.

3. Transfer the sample into a clean 1.5 mL microcentrifuge tube.

4. Add 5 volumes CP Buffer.

   Note: Volume refers to the size of your PCR reaction. For example, if your PCR reaction is 50 µL, you would use 250 µL CP Buffer.

5. Vortex to mix thoroughly. Briefly centrifuge to collect any drops from the inside of the lid.

6. Insert a MicroElute® DNA Mini Column into a 2 mL Collection Tube (provided).

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the MicroElute® DNA Mini Column.
2. Centrifuge at 10,000 x *g* for 30 seconds.
3. Add 500 µL sterile deionized water to the MicroElute® DNA Mini Column.
4. Centrifuge at 10,000 x *g* for 30 seconds.
5. Discard the filtrate and reuse the collection tube.

7. Transfer the sample from Step 5 to the MicroElute® DNA Mini Column.

8. Centrifuge at maximum speed (≥13,000 x *g*) for 1 minute at room temperature.

9. Discard the filtrate and reuse collection tube.

10. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with ethanol before use. Please see the Preparing Reagents section on Page 4 for instructions.

11. Centrifuge at maximum speed for 1 minute.

12. Discard the filtrate and reuse collection tube.

13. Repeat Steps 10-12 for a second DNA Wash Buffer wash step.

14. Centrifuge the empty MicroElute® DNA Mini Column at maximum speed for 2 minutes to dry the column.

    Note: This step is critical for removal of trace ethanol that may interfere with downstream applications**.**

15. Transfer the MicroElute® DNA Mini Column into a clean 1.5 mL microcentrifuge tube (not provided).

16. Add 10-20 µL Elution Buffer, TE Buffer, or sterile deionized water directly to the center of column matrix.

17. Let sit at room temperature for 2 minutes.

18. Centrifuge at maximum speed for 1 minute.

    Note: This represents approximately 80-90% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

19. Store DNA at -20°C.

MicroElute® Cycle Pure Kit - Vacuum Protocol

Materials and Equipment to be Supplied by User:

- Vacuum Manifold
- Microcentrifuge capable of at least 13,000 x *g*
- Nuclease-free 1.5 mL microcentrifuge tubes
- 100% ethanol
- Optional: 3M NaOH
- Optional: Sterile deionized water or TE Buffer

Before Starting:

- Prepare DNA Wash Buffer according to the "Preparing Reagents" section on Page 4.

1. Perform agarose gel/ethidium bromide electrophoresis to analyze PCR product.

2. Determine the volume of your PCR reaction.

3. Transfer the sample into a clean 1.5 mL microcentrifuge tube.

4. Add 5 volumes CP Buffer.

   Note: Volume refers to the size of your PCR reaction. For example, if your PCR reaction is 50 µL, you would use 250 µL CP Buffer.

5. Vortex to mix thoroughly. Briefly centrifuge to collect any drops from the inside of the lid.

6. Prepare the vacuum manifold according to manufacturer's instructions and connect the MicroElute® DNA Mini Column to the manifold.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the MicroElute® DNA Mini Column.
2. Switch on vacuum source to draw the buffer through the column.
3. Add 500 µL sterile deionized water to the MicroElute® DNA Mini Column..
4. Switch on vacuum source to draw the water through the column.
5. Turn off the vacuum.

7. Transfer the entire sample from Step 5 to the MicroElute® DNA Mini Column.

8. Switch on vacuum source to draw the sample through the column.

9. Turn off the vacuum.

10. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with ethanol before use. Please see the Preparing Reagents section on Page 4 for instructions.

11. Switch on vacuum source to draw the DNA Wash Buffer through the column.

12. Turn off the vacuum.

13. Repeat Steps 10-12 for a second DNA Wash Buffer wash step.

14. Transfer the MicroElute® DNA Mini Column into a 2 mL Collection Tube (provided).

15. Centrifuge the empty MicroElute® DNA Mini Column at maximum speed for 2 minutes to dry the column.

    Note: This step is critical for removal of trace ethanol that may interfere with downstream applications**.**

16. Transfer the MicroElute® DNA Mini Column into a clean 1.5 mL microcentrifuge tube (not provided).

17. Add 10-20 µL Elution Buffer, TE Buffer, or sterile deionized water directly to the center of column matrix.

18. Let sit at room temperature for 2 minutes.

19. Centrifuge at maximum speed for 1 minute.

    Note: This represents approximately 80-90% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

20. Store DNA at -20°C.

MicroElute® Gel Extraction Kit - Centrifugation Protocol

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x *g*
- 100% ethanol
- 1.5 mL microcentrifuge tubes
- Incubator capable of 55°C
- Vortexer
- Optional: 3M NaOH
- Optional: Sterile deionized water

Before Starting:

- Prepare SPW Wash Buffer according to "Preparing Reagents" section on Page 4.

1. Perform agarose gel/ethidium bromide electrophoresis to fractionate DNA fragments. Any type or grade of agarose may be used. However, it is strongly recommended that fresh TAE buffer or TBE buffer be used as running buffer. Do not reuse running buffer as its pH will increase and reduce yields.

2. When adequate separation of bands has occurred, carefully excise the DNA fragment of interest using a wide, clean, sharp scalpel. Minimize the size of the gel slice by removing extra agarose.

3. Determine the appropriate volume of the gel slice by weighing it in a clean 1.5 mL microcentrifuge tube. Assuming a density of 1 g/mL, the volume of gel is derived as follows: a gel slice of mass 0.3 g will have a volume of 0.3 mL.

4. Add 1 volume Binding Buffer (XP2).

5. Incubate at 60°C for 7 minutes or until the gel has completely melted. Vortex or shake the tube every 2-3 minutes.

Important: Monitor the pH of the Gel/Binding Buffer mixture after the gel has completely dissolved. DNA yields will significantly decrease when the pH > 8.0. If the color of the mixture becomes orange or red, add 5 µL 5M sodium acetate (pH 5.2) to bring the pH down. After this adjustment, the color of the Gel/Binding Buffer mixture should be light yellow.

6. Insert a MicroElute® DNA Mini Column in a 2 mL Collection Tube.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the MicroElute® DNA Mini Column.
2. Centrifuge at 10,000 x *g* for 30 seconds.
3. Add 500 µL sterile deionized water to the MicroElute® DNA Mini Column.
4. Centrifuge at 10,000 x *g* for 30 seconds.
5. Discard the filtrate and reuse the collection tube.

7. Transfer no more than 700 µL DNA/agarose solution from Step 5 to the MicroElute® DNA Mini Column.

   Note: Each MicroElute® DNA Mini Column has a total capacity of 10 µg DNA. If the expected yield is larger, divide the sample into the appropriate number of columns.

8. Centrifuge at 10,000 x *g* for 1 minute at room temperature.

9. Discard the filtrate and reuse collection tube.

10. Repeat Steps 7-9 until all of the sample has been transferred to the column.

11. Add 300 µL Binding Buffer (XP2).

12. Centrifuge at maximum speed (≥13,000 x *g*) for 30 seconds at room temperature.

13. Discard the filtrate and reuse collection tube.

14. Add 700 µL SPW Wash Buffer.

    Note: SPW Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

15. Centrifuge at maximum speed for 1 minute at room temperature.

16. Discard the filtrate and reuse collection tube.

Optional: Repeat Steps 14-16 for a second SPW Wash Buffer wash step. Perform the second wash step for any salt sensitive downstream applications.

17. Centrifuge the empty MicroElute® DNA Mini Column for 2 minutes at maximum speed to dry the column matrix.

    Note: It is important to dry the MicroElute® DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

18. Transfer the MicroElute® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

19. Add 10-20 µL Elution Buffer or sterile deionized water directly to the center of the column membrane.

    Note: The efficiency of eluting DNA from the MicroElute® DNA Mini Column is dependent on pH. If eluting DNA with sterile deionized water, make sure that the pH is around 8.5.

20. Let sit at room temperature for 2 minutes.

21. Centrifuge at maximum speed for 1 minute.

    Note: This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

22. Store DNA at -20°C.

MicroElute® Gel Extraction Kit - Vacuum Protocol

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x *g*
- 100% ethanol
- 1.5 mL microcentrifuge tubes
- Incubator capable of 55°C
- Vacuum Manifold
- Vortexer
- Optional: 3M NaOH
- Optional: Sterile deionized water

Before Starting:

- Prepare SPW Wash Buffer according to "Preparing Reagents" section on page 4.

1. Perform agarose gel/ethidium bromide electrophoresis to fractionate DNA fragments. Any type or grade of agarose may be used. However, it is strongly recommended that fresh TAE buffer or TBE buffer be used as running buffer. Do not reuse running buffer as its pH will increase and reduce yields.

2. When adequate separation of bands has occurred, carefully excise the DNA fragment of interest using a wide, clean, sharp scalpel. Minimize the size of the gel slice by removing extra agarose.

3. Determine the appropriate volume of the gel slice by weighing it in a clean 1.5 mL microcentrifuge tube. Assuming a density of 1 g/mL, the volume of gel is derived as follows: a gel slice of mass 0.3 g will have a volume of 0.3 mL.

4. Add 1 volume Binding Buffer (XP2).

5. Incubate at 60°C for 7 minutes or until the gel has completely melted. Vortex or shake the tube every 2-3 minutes.

6. Prepare the vacuum manifold according to manufacturer's instructions.

7. Connect the MicroElute® DNA Mini Column to the vacuum manifold.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the MicroElute® DNA Mini Column.
2. Switch on vacuum source to draw the buffer through the column.
3. Add 500 µL sterile deionized water to the MicroElute® DNA Mini Column..
4. Switch on vacuum source to draw the water through the column.
5. Turn off the vacuum.

8. Add no more than 700 µL DNA/agarose solution from Step 5 to the MicroElute® DNA Mini Column.

9. Turn on the vacuum source to draw the sample through the column.

10. Turn off the vacuum.

11. Repeat Steps 8-10 until all of the sample has been transferred to the column.

12. Add 300 µL Binding Buffer (XP2).

13. Turn on the vacuum source to draw the sample through the column.

14. Turn off the vacuum.

15. Add 700 µL SPW Wash Buffer.

    Note: SPW Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

16. Turn on the vacuum source to draw the sample through the column.

17. Turn off the vacuum.

18. Repeat Steps 15-17 for a second DNA Wash Buffer wash step.

19. Transfer the MicroElute® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

20. Centrifuge the empty MicroElute® DNA Mini Column for 2 minutes at maximum speed to dry the column matrix.

    Note: It is important to dry the MicroElute® DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

21. Transfer the MicroElute® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

22. Add 10-20 µL Elution Buffer or sterile deionized water directly to the center of the column membrane.

    Note: The efficiency of eluting DNA from the MicroElute® DNA Mini Column is dependent on pH. If eluting DNA with sterile deionized water, make sure that the pH is around 8.5.

23. Let sit at room temperature for 2 minutes.

24. Centrifuge at maximum speed for 1 minute.

    Note: This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

25. Store DNA at -20°C.

MicroElute® DNA Clean Up Kit - Centrifugation Protocol

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x *g*
- 100% ethanol
- 1.5 mL microcentrifuge tubes
- Vortexer
- Optional: 3M NaOH
- Optional: Sterile deionized water or TE buffer

Before Starting:

- Prepare SPW Wash Buffer according to "Preparing Reagents" section on Page 4.

1. Determine the volume of the enzymatic reaction.

2. Transfer the sample to a clean 1.5 mL microcentrifuge tube.

3. Add 3 volumes DP Buffer. Vortex to mix thoroughly.

   Note: For example, add 300 µL DP Buffer to 100 µL enzymatic reaction.

4. Insert a MicroElute® DNA Mini Column in a 2 mL Collection Tube.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the MicroElute® DNA Mini Column.
2. Centrifuge at 10,000 x *g* for 30 seconds.
3. Add 500 µL sterile deionized water to the MicroElute® DNA Mini Column.
4. Centrifuge at 10,000 x *g* for 30 seconds.
5. Discard the filtrate and reuse the collection tube.

5. Transfer the sample from Step 3 to the MicroElute® DNA Mini Column.

6. Centrifuge at maximum speed (≥13,000 x *g*) for 1 minute at room temperature.

7. Discard the filtrate and reuse collection tube.

8. Add 700 µL SPW Wash Buffer.

   Note: SPW Wash Buffer must be diluted with ethanol before use. Please see the Preparing Reagents section on Page 4 for instructions.

9. Centrifuge at maximum speed for 30 seconds.

10. Discard the filtrate and reuse collection tube.

11. Repeat Steps 8-10 for a second SPW Wash Buffer wash step.

12. Centrifuge the empty MicroElute® DNA Mini Column at maximum speed for 2 minutes to dry the column.

    Note: This step is critical for removal of trace ethanol that may interfere with downstream applications**.**

13. Transfer the MicroElute® DNA Mini Column into a clean 1.5 mL microcentrifuge tube (not provided).

14. Add 10-20 µL Elution Buffer, TE Buffer, or sterile deionized water directly to the center of column matrix.

15. Let sit at room temperature for 1 minute.

16. Centrifuge at maximum speed for 1 minute.

    Note: This represents approximately 80-90% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

17. Store DNA at -20°C.

MicroElute® DNA Clean Up Kit - Vacuum Protocol

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x *g*
- Vacuum Manifold
- 100% ethanol
- 1.5 mL microcentrifuge tubes
- Vortexer
- Optional: 3M NaOH
- Optional: Sterile deionized water or TE buffer

Before Starting:

- Prepare SPW Wash Buffer according to "Preparing Reagents" section on Page 4.

1. Determine the volume of the enzymatic reaction.

2. Transfer the sample to a clean 1.5 mL microcentrifuge tube.

3. Add 3 volumes DP Buffer. Vortex to mix thoroughly.

   Note: For example, add 300 µL DP Buffer to 100 µL enzymatic reaction.

4. Prepare the vacuum manifold according to manufacturer's instructions.

5. Connect the MicroElute® DNA Mini Column to the vacuum manifold.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the MicroElute® DNA Mini Column.
2. Switch on vacuum source to draw the buffer through the column.
3. Add 500 µL sterile deionized water to the MicroElute® DNA Mini Column..
4. Switch on vacuum source to draw the water through the column.
5. Turn off the vacuum.

6. Transfer the sample from Step 3 to the MicroElute® DNA Mini Column.

7. Turn on the vacuum source to draw the sample through the column.

8. Turn off the vacuum.

9. Add 700 µL SPW Wash Buffer.

   Note: SPW Wash Buffer must be diluted with ethanol before use. Please see the Preparing Reagents section on Page 4 for instructions.

10. Turn on the vacuum source to draw the buffer through the column.

11. Turn off the vacuum.

12. Repeat Steps 9-11 for a second SPW Wash Buffer wash step.

13. Centrifuge the empty MicroElute® DNA Mini Column at maximum speed for 2 minutes to dry the column.

    Note: This step is critical for removal of trace ethanol that may interfere with downstream applications**.**

14. Transfer the MicroElute® DNA Mini Column into a clean 1.5 mL microcentrifuge tube (not provided).

15. Add 10-20 µL Elution Buffer, TE Buffer, or sterile deionized water directly to the center of column matrix.

16. Let sit at room temperature for 1 minute.

17. Centrifuge at maximum speed for 1 minute.

    Note: This represents approximately 80-90% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

18. Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
Low DNA Yields	Not enough CP Buffer added to sample	Add more CP Buffer as indicated. For DNA fragments < 200 bp in size, add up to 6 volumes of CP Buffer.
	pH of the sample mixture is too high	Add 10-20 µL sodium acetate (pH 5.2) to the sample and mix.
Problem	Cause	Solution
Clogged Column in Gel Extraction	Incompletely dissolved gel	• Increase incubation time. • Increase Binding Buffer (XP2) volume.
No DNA Eluted	SPW Wash Buffer or DNA Wash Buffer was not diluted with ethanol	Prepare SPW Wash Buffer or DNA Wash Buffer as instructed on Page 4.
Optical densities do not agree with DNA yield on agarose gel	Trace contaminants eluted from column increase A 260	Wash column as instructed. Alternatively, rely on agarose gel/ ethidium bromide electrophoresis for quantification.
DNA sample floats out of well while loading agarose gel	Ethanol not removed completely from column following wash steps	Centrifuge column as instructed to dry before proceeding to elution.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
CP Buffer, 200 mL	PDR042
Binding Buffer (XP2), 200 mL	PDR040
DP Buffer, 100 mL	PDR047
Elution Buffer, 100 mL	PDR048
DNA Wash Buffer, 100 mL	PS010
MicroElute® DNA Mini Columns, 50 columns	MEDNACOL-01
SPW Wash Buffer, 25 mL	PDR045
2 mL Collection Tubes, 500/pk, 10 pk/cs	SS1-1370-00
1.5 mL DNase/RNase-free Microcentrifuge Tubes, 500/pk, 10 pk/cs	SS1-1210-00

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Qiagen®, QIAvac® and Vacman® are all trademarks of their respective companies.
PCR is a patented process of Hoffman-La Roche. Use of the PCR process requires a license.